AMERICAN JOURNAL OF PHARMACY

PUBLISHED MONTHLY BY THE

Philadelphia College of Pharmacy

VOL. 89 APRIL, 1917 NO. 4

CONTENTS

Terms, $3.00 per Annum, in advance. Issued in Monthly numbers of not less than 48 pages. Single Numbers, 30 Cents. Back Numbers, 50 Cents.

Address all communications to

The American Journal of Pharmacy, 145 North Queen Street, Lancaster, Pa., or 145 North Tenth Street, Philadelphia, Pa.

Entered as second-class matter February 21, 1917, at the Post Office at Lancaster, Pa., under the Act of March 3, 1879.

American Journal of Pharmacy

ESTABLISHED IN 1825

Four preliminary numbers were published at different times until in 1829, when the publication of the regular volumes began. Since then the publication has been uninterrupted. During the period from 1829 to 1852 four numbers were published annually, except in 1847, when five numbers were published. From 1853 to 1874 six numbers were published. Since this time twelve numbers have been published annually.

MANUSCRIPTS should be sent to the Editor. It should be stated in this connection that the Editor does not assume any responsibility in connection with the views or investigations of contributors, other than to exercise general care in the selection of matter.

Contributors are allowed a reasonable number of copies of this JOURNAL free of charge, if applied for when the proof is returned.

REPRINTS, if desired, must be applied for when the proof is returned. The table below shows the *approximate* cost of reprints, the make-up of the pages to be identical with the same as in the JOURNAL. The actual cost may vary from the figures given, as it will depend upon the amount of pressework, paper, binding, etc. Reprints containing half-tones may be expected to cost somewhat more than the rates given.

	2 pp.	4 pp.	8 pp.	16 pp.	COVERS WITH TITLE
25 copies	$2.25	$3.75	$7.75	$9.00	25 copies
50 "	2.50	4.00	8.25	9.75	50 "
100 "	2.75	4.25	9.00	10.75	100 "
250 "	3.75	4.75	10.00	12.00	250 "

THE AMERICAN
JOURNAL OF PHARMACY

APRIL, 1917

DIGITALIS THAPSI LIN.

By O. A. Farwell and H. C. Hamilton.

Late in 1916, samples of a new drug were offered in America under the name of Spanish digitalis. It has no general resemblance to the official digitalis; indeed, at first glance, it looks more like mullein than anything else, being yellowish gray or yellowish green in color.

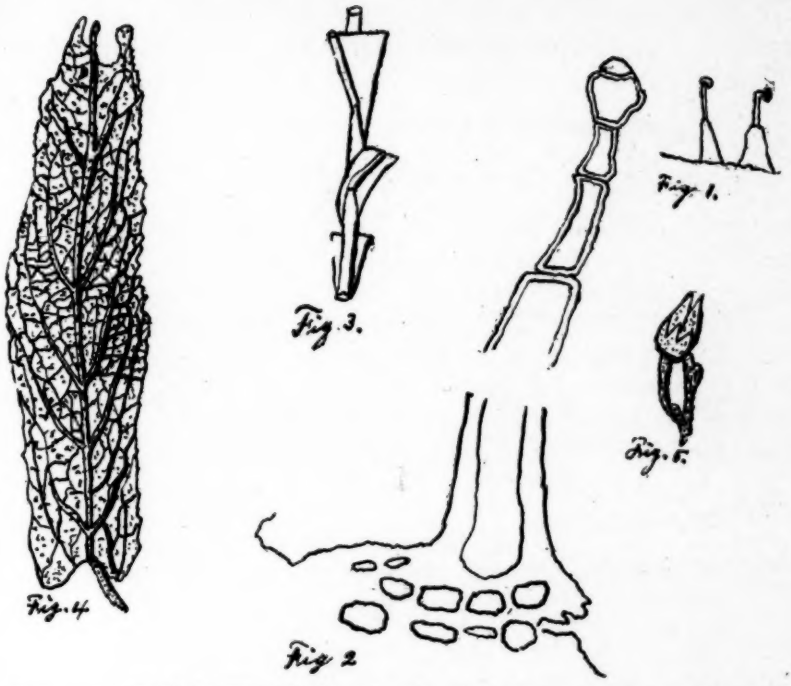
It has been thought advisable to investigate the histological and pharmacological aspects of the drug and the following papers are the result. Each author is responsible for his own section of the work.

HISTOLOGY.

By Oliver Atkins Farwell.

The drug consists of broken fragments of leaf (Fig. 4), stems (Fig. 3), and capsules (Fig. 5). Stems slender, terete or somewhat angular, green or purplish, densely covered with gland-tipped, 3–7 celled hairs (Figs. 1 and 2), ½ a millimeter or less in length, velvety not rough. Bases of alternate leaves show a slight, decurrent line (Fig. 3) on the stem; leaves in fragments, rarely entire, 2–3 cm. wide by 3–15 in length narrowly oblong or oblong-lanceolate, gradually tapering to a broad sessile base, margin coarsely denticulate; on the lower surface the midvein is prominent with 4–6 inconspicuous pairs of veins, rugose; both upper and lower surfaces are covered with glandular hairs but they are not so long as those found upon the stem, soft velvety, not rough. Flowers occasional, cylindrical, upper parts purplish, slightly pubescent externally; when expanded, cylindrical below, contracted just above the ovary and then

abruptly expanded into a tube much longer than the usually 5-lobed, slightly 2-lipped limb; stamens 4, didynamous, attached low down on the tube, included; calyx present, glandular, 5-parted, about ⅕ the length of the corolla, segments lanceolate. Capsules ovoid, about 15 Mm. in length, greenish and more or less glandular, to pale brownish or yellowish green, with only traces of the glandular indument, with fragments of the calyx at the base, on a slender pedicle, 3 Cm. or less in length, which is clavate under the capsule; fruit partially separating into two, one-celled, many-seeded sections, opening at the apex on the inner surface by a large pore. Odor, slight; taste, bitter and slightly acrid. This drug is not the official *Digitalis purpurea* Lin. as the leaves are sessile, somewhat decurrent, and of the same color on both sides. It may be the closely related *D. Thapsi* Lin. A cross-section of the upper portion of a stem, 2½ Mm. thick, shows a pith 1 Mm. in diameter surrounded by a circle of wood (Fig. 6, *D*), 0.3 Mm. wide and a bark about .5 Mm. The outer bark shows epidermal cells that are small (12–20 microns) with rather thick outer walls covered with a strongly papillate cuticle (Fig. 7, *A*); immediately under the epidermis is a layer (130 microns) of unlignified hypodermal cells 5–8 tiers in depth (Fig. 7, *B*); internally to this is a layer of parenchyma (Fig. 8), of equal depth but composed of fewer, 4–6 tiers of cells; the inner bark (Fig. 6) is made up of a continuous circle of bast (*A*) 130 microns deep and a narrower layer of sieve tissue (*B*). The cells of the pith (*E*) often reach a size of 70 microns in diameter, are slightly lignified or cutinized and non-porous. The stomata (Fig. 9) on the leaf occur in the ratio of about 8 to an area of 150 microns square. The epidermis is essentially the same as for the green bark of the stem. The palisade tissue is one cell in depth; the cells of the sponge tissue are well filled with oil. No crystals were detected. The midrib (Fig. 10) forms a very prominent keel on the dorsal (lower) surface of the leaf. The vascular strand is rather broadly ovate in outline, the phlœm passing almost completely around the xylem. On the ventral side there are several layers of collenchyma and on the dorsal side the greater part of the tissue consists of the water-storage-cells with a layer or two of collenchyma between it and the epidermis.



FIGS. 1–5. *Digitalis Thapsi* Lin., showing leaf fragments, a capsule and gland-tipped hairs.

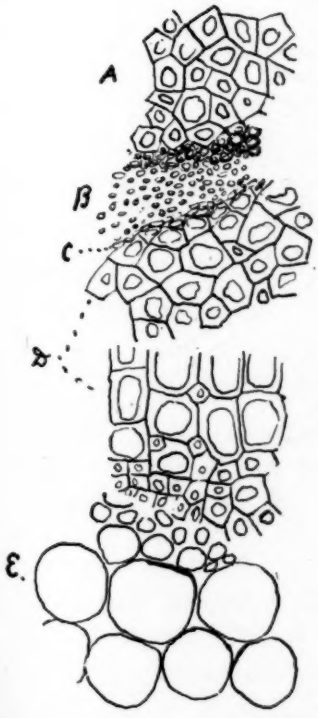
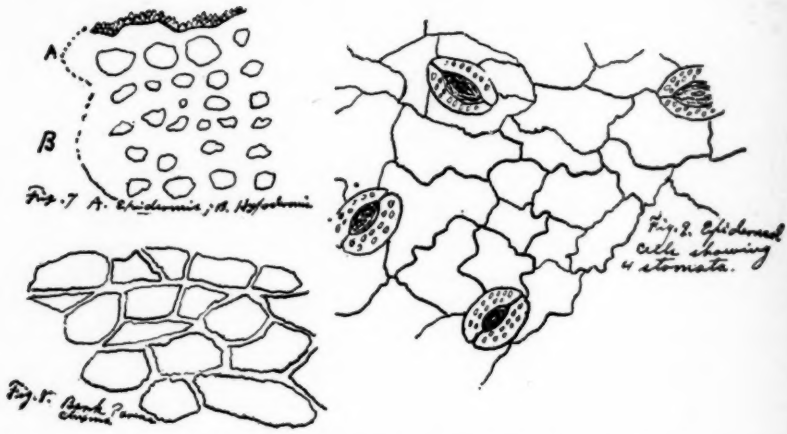


FIG. 6. Transverse section through portion of stem of *Digitalis Thapsi* Lin. *A*, bast fibers; *B*, sieve tissue; *C*, cambium; *D*, woody tissues; *E*, pith.



FIGS. 7–9. *Digitalis Thapsi* Lin., showing structure of stem and stomata on leaf.

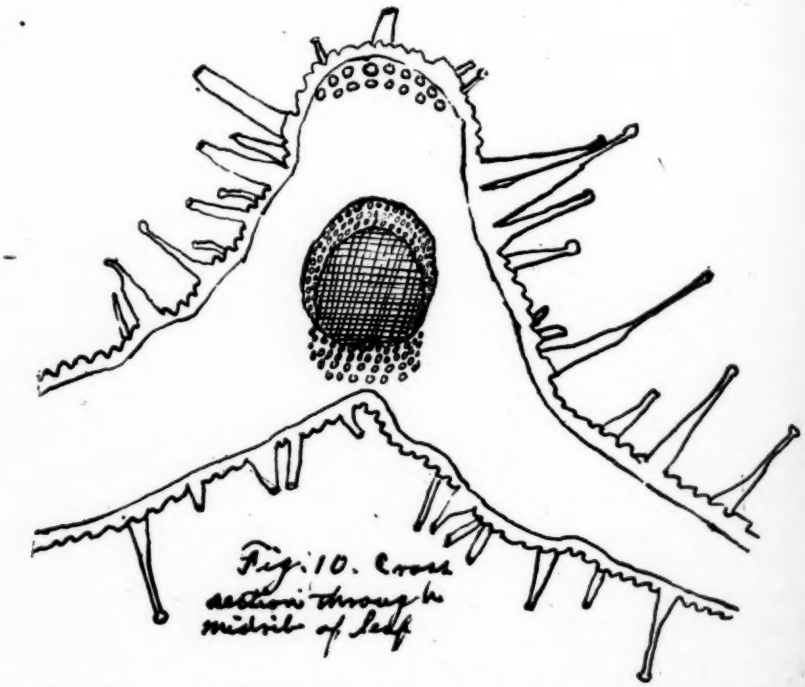


FIG. 10. Cross-section through midrib of leaf of *Digitalis Thapsi* Lin.

PHARMACOLOGIC ACTION.

By Herbert C. Hamilton.

A preliminary investigation of *Digitalis Thapsi* to determine whether it possessed any of the therapeutic properties of the official drug was carried out on frogs by the M. L. D. method for standardization.[1] The drug in tincture form was properly diluted and injected into frogs in gradually decreasing doses until the minimum was found which was sufficiently toxic to kill.

This dose was just one third that of a similar tincture from an average sample of official drug as shown below. Data of final tests only are here recorded.

Standard Tr. Digitalis.

Wt. of frog.	Dose per gm.	Total dose.
20	.009	18 alive
20	.010	20 dead
19	.010	19 dead
21	.011	23 dead
22	.011	23 dead

Tincture from Thapsi Drug.

18	.0025	23 alive
20	.0030	30 alive
20	.0035	35 dead
21	.0040	42 dead
22	.0045	49 dead

Dilution 1 in 5.

The above are the final tests showing the comparative toxicities as determined by the minimum lethal doses of the two tinctures which are:

Standard Tinct. Digitalis	0.010
Tinct. Digitalis Thapsi	0.0035

That the drug belongs to the digitalis series of heart tonics is shown by the fact that in every case the heart was found to have stopped in systole, *i. e.*, with the apex of the heart strongly contracted.

A qualitative test further to confirm the action of the drug was made by dropping the diluted tinctures on the laid-bare frog's heart. The two tinctures were compared as before.

[1] Houghton and Hamilton, Amer. Jour. of Pharm., October, 1909.

First Experiment.

Frog pithed and stretched out on board.

Normal heart rate.
9:15.....20 beats in 20 seconds.
9:16.....1 drop tinct. thapsi drug dropped on the laid-bare heart.
9:20.....20 beats in 22 seconds.
9:21.....20 beats in 23 seconds.
9:24.....20 beats in 26 seconds.
9:25.....2 drops, same solution.
9:28.....20 beats in 30 seconds.
9:30.....20 beats in 40 seconds.
9:35.....Beats irregular and indistinct.
9:40.....Stopped in systole.

Second Experiment.

Frog pithed and fastened to board.

Normal heart rate.
10:30.....20 beats in 24 seconds.
10:35.....2 drops tr. digitalis U.S.P.
10:37.....20 beats in 28 seconds.
10:39.....20 beats in 30 seconds.
10:45.....20 beats in 30 seconds.
10:46.....2 drops, same solution.
10:50.....20 beats in 35 seconds.
10:55.....20 beats in 40 seconds.
11:00.....2 drops, same solution.
11:05.....20 beats in 50 seconds.
11:10.....20 beats in 55 seconds.
Irregular beats and final stoppage in systole.

Third Experiment.

Normal heart rate.
1:35.....20 beats in 20 seconds.
1:40.....2 drops thapsi drug diluted, 1 in 3 from the tincture.
1:45.....20 beats in 22 seconds.
1:47.....20 beats in 23 seconds.
2 drops, same solution.
1:50.....20 beats in 28 seconds, very powerful.
1:54.....20 beats in 30 seconds.
1:55.....2 drops, same solution.
1:57.....20 beats in 33 seconds.
2:00.....20 beats in 50 seconds.
Very irregular.
2:15.....20 beats in 200 seconds, stopped in systole.

These experiments show the typical action of the drug in slowing and strengthening the heart beat to be identical with that of the U. S. P. digitalis. The powerful ventricular contractions were especially noticeable.

The other typical heart-tonic action of digitalis, namely, its pressor effect on the circulatory system, is observed best on an anesthetized dog. The dog is anesthetized with chloreton[2] which affects the system only slightly. These experiments failed to discover this typical effect when thapsi drug was administered while the tracings obtained after injection of the official drug almost invariably show a distinct rise in blood pressure followed, unless the dose be too large, by a return approximately to normal.

The two drugs were injected one following the other into two dogs. The first dog received first 0.2 Cc. of the tincture (freed from alcohol) of the thapsi drug. The rate was lowered, but no increase of blood pressure could be detected. After the action of the drug was apparently over, about one hour after first injection, 0.5 Cc. tr. digitalis, U.S.P. was administered followed shortly by the characteristic effects of the drug, including a distinct increase in blood pressure. The heart-beats soon became irregular and the dog died.

In another dog the order of dosing was reversed, the official tincture being administered first followed by an equivalent dose of the thapsi tincture. The effects observed from the first experiment were duplicated in the second, but reversed. The official tincture raised the blood pressure while the thapsi tincture had no effect on the blood pressure, but slowed the heart and the experiment ended by the irregular heart beats and final death of the animal.

While the experiments detailed above are more or less preliminary to a more exhaustive examination of the pharmacologic properties of this variety of digitalis, they are sufficient to show that it possesses at least two of the valuable properties of *Digitalis purpurea,* namely, the effects on the rate and amplitude of the heart beat. They show also that it more nearly resembles strophanthus in having no effect or at least very slight action on the blood pressure[3]—a property which in many cases is a distinct advantage.

The observed activity of the drug—a toxicity three times as great as that of the average official variety—is not to be taken as repre-

[2] Rowe, *Journal of Pharmacology and Experimental Therapeutics,* vol. 9, 1916.

[3] Cushney, "Pharmacology and Therapeutics."

senting the activity of this variety. It represents that of only one sample and does not exceed that of occasional samples of American grown *Digitalis purpurea*. Further work and a number of samples would be required to determine its average activity.

That this drug may become a valuable adjunct to the repertoire of heart tonics is evident from its similarity in action both to the official variety of digitalis and to strophanthus, which latter is becoming more and more generally used in therapy.

A more extended pharmacologic research is planned for the near future.

DEPARTMENTS OF BOTANY AND
PHARMACOLOGY, PARKE, DAVIS & CO.,
DETROIT, MICH.

RAPID APPROXIMATE DETERMINATION OF MILK SUGAR IN HEADACHE POWDERS.

BY REGINALD MILLER.

This method depends upon the fact that milk sugar when heated with ammonium hydroxide gives a yellow to red color[1] the intensity of which is used as a measure of the amount present.

Take a weighed portion of the powder (about 1 g.), transfer to a small beaker and extract[2] repeatedly with a mixture[3] of chloroform and absolute alcohol, by pouring about 12 mils of the mixture upon the powder, stirring with a glass rod, allowing to settle and then decanting the solution through a small filter paper, after the extraction

[1] The color produced is yellow when about .005 g. is present, and pinkish red in the presence of about .025 g. of milk sugar. On the addition of water (making volume up to 50 mils) the pinkish color fades to yellow after standing for five minutes.

A color similar to that obtained from .025 g. of milk sugar is produced by maltose, while dextrose and levulose produce a dark yellow, mannose a light yellow, and with cane sugar the solution remains colorless. Upon dilution to 50 mils with water, and after standing five minutes the depth of color corresponding to that obtained from .005 g. of milk sugar is produced by about .005 g. of maltose; .020 g. of dextrose or levulose or .050 g. of mannose.

[2] About six extractions are generally sufficient; in many cases the method may be applied directly by treating one gram of the powder with sufficient water to make 100 mils and then making the determination. Sodium bicarbonate does not interfere with the determination.

[3] This mixture consists of two volumes of chloroform and one volume of absolute alcohol, and is used to remove acetanilid, phenacetin, salol, etc.

is complete, transfer the filter paper and residue to the beaker, add 25 mils of hot distilled water to dissolve the milk sugar; transfer this solution to a 100 mil volumetric flask, wash the filter paper and beaker with more water until the collected washings total 100 mils. Cool to room temperature and add water to the 100 mil mark.

Portions of this aqueous solution corresponding in volume to the amounts used in the standard tubes are measured into Nessler tubes and treated exactly like the standards.

Standards are prepared as follows:

Dissolve .500 g. of milk sugar in sufficient water to make 100 mils; one mil of this solution contains .005 g. of milk sugar.

Tube[4] *A* — 1 mil of st. sol. = (.005 g. milk sugar) + 4 mils of water + 10 mils of conc. ammonium hydroxide.

Tube *B* + 2 mils of st. sol. = (.010 g. milk sugar) + 3 mils of water + 10 mils of conc. ammonium hydroxide.

Tube *C* + 3 mils of st. sol. = (.015 g. milk sugar) + 2 mils of water + 10 mils of conc. ammonium hydroxide.

Tube *D* + 4 mils of st. sol. = (.020 g. milk sugar) + 1 mil of water + 10 mils of conc. ammonium hydroxide.

Tube *E* + 5 mils of st. sol. = (.025 g. milk sugar) + 10 mils of conc. ammonium hydroxide.

These tubes as well as those containing the unknown are placed in a water bath and heated to about 95° C. for about half hour, the volume in each tube is then made to 50 mils and allowed to stand 5 minutes. The depth of color produced in the tubes containing the sample is compared with the standards until two are found (one standard and one unknown) that correspond. Computations are then made from the standard tube.

Chemical Laboratory,
Department of Health, City of New York.

APPROXIMATE DETERMINATION OF NOVASPIRIN, ALONE OR WHEN MIXED WITH ASPIRIN.

By Reginald Miller.

This method depends upon the fact that sodium hydrate produces a yellow color with novaspirin but remains colorless with aspirin.[1]

[4] Nessler tubes holding 100 mils are used.

[1] The aspirin must not be present in a greater proportion than two parts of aspirin to one part of novaspirin, if present in a larger amount, it interferes with the production of the yellow color.

The intensity of color produced is used as the measure of the quantity of novaspirin present.

Dissolve from .200 g. to .500 g. of powder (containing novaspirin or a mixture of novaspirin and aspirin) in 25 mils of 95 per cent. alcohol; add sufficient water to make 50 mils. Measure portions of this solution into Nessler tubes and treat as directed below in preparation of standards.

Standards are prepared in Nessler tubes as follows: A solution is made by dissolving .100 g. of novaspirin in 25 mils of alcohol and then adding sufficient water to make 50 mils.

One mil of this solution contains .002 g. of novaspirin.

Tube[2] *A* — 1 mil of st. sol. + 25 mils of water + 2 mils of *N*/5 sodium hydroxide = .002 g. novaspirin.

Tube *B* — 2 mils of st. sol. + 25 mils of water + 2 mils of *N*/5 sodium hydroxide = .004 g. novaspirin.

Tube *C* — 3 mils of st. sol. + 25 mils of water + 2 mils of *N*/5 sodium hydroxide = .006 g. novaspirin.

Tube *D* — 4 mils of st. sol. + 25 mils of water + 2 mils of *N*/5 sodium hydroxide = .008 g. novaspirin.

After the addition of the water and sodium hydroxide, more water is added to each tube, making a volume of 50 mils.

The tubes containing the standards are compared after an elapse of 5 minutes with those containing the samples until two are found which correspond in depth of color (one standard tube and one containing the sample). Computations are then made using the standard tube as a basis.[3]

CHEMICAL LABORATORY,
DEPARTMENT OF HEALTH, CITY OF NEW YORK.

RAPID APPROXIMATE DETERMINATION OF PHENACETIN WHEN MIXED WITH ACETANILID.

BY REGINALD MILLER.

This method is based upon the well-known nitric acid[1] test for phenacetin, which gives an intense yellow to orange-red color, and

[2] More satisfactory results are obtained by following the procedure outlined in this table. A more staple color is produced this way.

[3] The readings should be made after an elapse of about five minutes but before an elapse of fifteen minutes when the color gradually fades and is untrustworthy.

[1] Autenreith-Hinsberg Test, *Archiv der Pharmacie*, Band 229, 456 (1891).

also upon the facts that phenacetin is soluble in methyl alcohol and the addition of nitric acid to such a solution properly diluted gives a yellow color, the intensity of which is used as a measure of the phenacetin present by comparison in Nessler tubes with standards.

Standards are prepared as follows: A standard solution is made by dissolving .500 g. of phenacetin in sufficient methyl alcohol[2] to make 100 mils; one mil of this solution then contains .005 g. of phenacetin.

Tube *A* — 1 mil of st. sol. + 4 mils methyl alcohol = .005 g. phenacetin
Tube *B* — 2 mils of st. sol. + 3 mils methyl alcohol = .010 g. phenacetin
Tube *C* — 3 mils of st. sol. + 2 mils methyl alcohol = .015 g. phenacetin
Tube *D* — 4 mils of st. sol. + 1 mil methyl alcohol = .020 g. phenacetin
Tube *E* — 5 mils of st. sol. + methyl alcohol = .025 g. phenacetin

To each tube except the last, methyl alcohol is added as indicated, in order to have an equal amount in each tube,[3] then 5 mils of water and 3 mils of concentrated nitric acid is added to each tube.[4]

A qualitative test is made on the sample and this gives an idea as to the amount of phenacetin present. A definite amount of the sample is dissolved in methyl alcohol, so that one mil of the solution will contain between .005 g. and .025 g. of phenacetin. Portions of this solution are then measured into Nessler tubes and treated the same as the standards all of which must be made at the same time. After an elapse of 5 minutes make up the volume in each tube to 50 or 100 mils and compare the intensity of color in the tubes containing the sample, with the color produced in the tubes containing the standards until two are found (one standard and one unknown) that correspond. Computations are then made using the standard tube as a basis.

Chemical Laboratory,
Department of Health, City of New York.

[2] Pure methyl alcohol is used (reagent).

[3] This is necessary because the methyl alcohol decreases the intensity of color produced.

[4] It is preferable to use a burette to measure the nitric acid.

SOME EXPERIMENTS ON THE CHEMICAL REACTIONS OF DIPHTHERIA ANTITOXIN.

By Albert C. Crawford and Carlton L. Andrus.[1]

Many of us expect to find that future advances in rational therapeutics will be made along chemotherapeutic lines and by following the methods used by nature, *i. e.*, by the use of antitoxins, etc. Hence it is essential to know something as to the chemistry of the antitoxins. From this point of view, we recently reviewed the literature on the chemistry of diphtheria antitoxin.[2] As a result of this summary, it becomes evident that there are two views; one is, that the antitoxin is not necessarily a globulin, but is carried down with them on precipitation; the other view, held by most workers, is that diphtheria antitoxin is a globulin, and some uncorroborated work even suggests that ordinary egg globulin could be converted into diphtheria antitoxin.

We have been carrying on experiments to determine the reaction of diphtheria antitoxin to various reagents, and assuming it were not a globulin, to find whether it could be separated from the globulins. No doubt the response to reagents will vary, according to the solution in which the antitoxin occurs.

For part of this work we have used unconcentrated preparations obtained through the courtesy of the Cutter Laboratory at Berkeley. The first preparation contained over 500 antitoxic units to 1 Cc. It was prepared by heating a mixture of 70 parts of diluted antitoxic serum with 30 parts of saturated ammonium sulphate solution, *i. e.*, Banzhaf's method. The second preparation was simply a solution of the precipitate from antitoxic serum by from 30 to 50 per cent. saturation of ammonium sulphate. It contained 450 units to 1 Cc. The third preparation was a concentrated globulin solution (40,000 units in about 13.5 Cc.). This was prepared by a modified Banzhaf method and was given us by Parke Davis & Co. The fourth, was a globulin preparation from the Cutter Laboratory and contained 250 units per 1 Cc.

[1] The expense of this work was partly covered by a grant from the Committee on Therapeutic Research of the Council of Pharmacy and Chemistry of the American Medical Association.

[2] Crawford, A. C., and Foster, M. G., *Biochem. Bull.*, 1917, vol. 6, p. 1.

The determinations of the antitoxic values were made by the firms from which the preparations were obtained and controlled, when any variation was suspected. The toxin was made by the Cutter Laboratory and its L + dose determined there, but was checked with standard antitoxin obtained from Dr. G. McCoy of the Hygienic Laboratory.

In these experiments usually only three guinea pigs were used for each test. The injections were made subcutaneously in the mid-abdominal region and each injection had a volume of 4.5 Cc.[3] All evaporations were done in vacuo between 50° and 55° C., and preparations which were not used immediately were usually kept in an ice box. Instead of filtering, solutions were obtained by centrifugalizing at 4,000 revolutions a minute.

The guinea pigs weighed about 250 Gm., although owing to difficulty in obtaining a proper supply, guinea pigs below 250 Gm. were often used. At the autopsy of those dying after the injections, enlargement and hemorrhages into the suprarenal glands, hemorrhages in the gastric mucosa and signs of local irritation were the only macroscopic changes looked for and found No histological examinations were made.

Some of globulin preparation IV was evaporated to dryness in vacuo, and the flask jarred with a vibrator for varying intervals of time from July 20 to July 31, 1914, then put aside till February, 1917, but no signs of crystallization have appeared.

Fifteen Cc. of globulin II were shaken with 400 Mg. cholesterin and centrifugalized. The fluid retained its full antitoxic value.

After precipitation of antitoxin with aluminium hydroxide[4] the filtrate of globulin preparation IV became inactive.

Five Cc. of globulin preparation I were evaporated to dryness and then twice extracted at room temperature with 20 Cc. acetic ether c.p. The undissolved portion possessed about the antitoxic value of the original, showing the antitoxin to be insoluble in acetic ether.

Five Cc. of globulin preparation I were evaporated to dryness then extracted with 20 Cc. *n*/10 NaOH and after a few minutes neutralized with 20 Cc. *n*/10 HCl. Even 3 times the theoretical amount which should neutralize one L + dose failed to protect. Evidently *n*/10 NaOH injures this antitoxin.

The same amount of this globulin was evaporated and treated

[3] Rosenau, M. J., Hyg. Lab. Bull. 21, 1905.

[4] *Journ. Amer. Chem. Soc.*, 1913, p. 820.

with 20 Cc. of *n*/100 NaOH, and after 10 minutes was neutralized with 20 Cc. of HCl *n*/100. This solution retained the full antitoxic value of the original, *i. e.*, short contact with *n*/100 NaOH does not injure antitoxin. No attempt was made to find if prolonged contact would injure it.

Five Cc. of globulin I were treated with 5 Cc. NaOH *n*/10, then neutralized with 5 Cc. HCl *n*/10. This was diluted to 150 Cc. 2 Cc. of this solution protected against one L+ dose of the toxin. Apparently there was some slight loss in activity.

Five Cc. globulin II was diluted with 5 Cc. NaCl (0.85 per cent.), treated with 10 Cc. NaOH *n*/10 and neutralized with 10 Cc. HCl *n*/10. This had about the full antitoxic value, *i. e.*, *n*/20 NaOH does not injure the antitoxin, at least in this preparation.

Ten Cc. globulin preparation II were treated with 10 Cc. NaOH *n*/10 and shaken with benzol. The benzol residue was shaken with 25 Cc. NaCl (0.85 per cent.) Even 3 Cc. did not protect against one L+ dose of the toxin. The mother fluid contained the full antitoxic value, showing that *n*/20 NaOH did not injure this antitoxin, and that it would not shake into alkaline benzol.

Ten Cc. of globulin II were treated with 5 Cc. NaOH *n*/10 and shaken with ether. The ether residue contained no antitoxin, while the mother fluid had its full value, *i. e.*, antitoxin is not soluble in alkaline ether.

Five Cc. of globulin preparation I were evaporated to dryness in vacuo and extracted twice with 20 Cc. of methyl alcohol (Merck). This alcohol was evaporated and the residue left by its evaporation was extracted with 10 Cc. normal NaCl. Even 3 Cc. of this did not protect from one L+ dose of the toxin. Presumably no antitoxin was present.

The residue after methyl extraction was dissolved with 20 Cc. of NaCl (0.85 per cent.) and 10 Cc. *n*/10 NaOH. It did not dissolve in NaCl. It was then neutralized with 10 Cc. *n*/10 HCl. This solution had the full antitoxic value of the original solution. The diphtheria antitoxin as present in this preparation is insoluble in methyl alcohol.

The same amount of preparation II was evaporated to dryness and treated with methyl alcohol containing the same amount of NaOH as used in the above test, but as very small amounts seemed to be dissolved, no tests were made on animals.

Five Cc. of preparation II were treated with 5 Cc. of *n*/10 NaOH

and 40 Cc. methyl alcohol (Merck), and after standing about 20 to 30 minutes were centrifugalized. The precipitate was dissolved in 25 Cc. *n*/100 NaOH and then neutralized with the same amount of *n*/100 HCl. This gave a solution much the color of the original globulin preparation, although the precipitate was only slightly colored. This solution was slightly less active than the original preparation, perhaps due to standing, as several days elapsed before we were able to test this preparation.

10 Cc. of globulin preparation I were diluted with 10 Cc. of NaCl (0.85 per cent.) and precipitated with a solution of lead subacetate, drop by drop, from a burette. The preparation was then centrifugalized and the supernatant fluid precipitated with sodium hydrogen phosphate, centrifugalized and then diluted to 150 Cc. Even 3 Cc. did not protect against one L + dose of the toxin.

Several other attempts were made to free antitoxin from proteins by means of lead subacetate solution, but in most cases the filtrate when freed from lead was inactive. In one case it possessed slight antitoxic value, but in this case a possible excess of the alkaline lead solution may explain the result, *i. e.*, solubility of antitoxin or globulin in weak alkali. In this latter case the lead filtrate contained a few antitoxic units, yet produced no anaphylactic reaction in a guinea pig.

10 Cc. of globulin preparation I were diluted with 10 Cc. of sodium chloride (0.85 per cent.) and precipitated with a cold saturated aqueous solution of picrolonic acid by means of a burette, then centrifugalized. The centrifugalized fluid was shaken with acetic ether to remove picrolonic acid, at least partly, although acetic ether did not seem to us as suitable for this purpose as isobutyl alcohol. After separating the undissolved acetic ether, the solution was diluted to an arbitrary amount (250 Cc.) Even 3 Cc. of this solution failed to protect against one L + dose of the toxin. The precipitate was shaken with NaCl .85 per cent. and made into a colloidal suspension of 250 Cc. Only 1 to 3 Cc. were tested. 1 Cc. of this suspension protected against one L + dose of the toxin, showing that most and perhaps all of the antitoxin was in the picrolonic acid precipitate.

A similar preparation was also precipitated with picrolonic acid. The precipitate was shaken into a colloidal solution or suspension with distilled water. This was centrifugalized and the sediment shaken with NaCl (0.85 per cent.). The H_2O solution was diluted

to 250 Cc. One to three Cc. were tested. One Cc. protected against one L + dose. Presumably the neutralizing power was even greater than shown. The colloidal solution obtained with 0.85 per cent. NaCl was diluted to 350 Cc. Even one Cc. protected against one L + dose of the toxin. Some antitoxin went into both preparations.

Three Cc. of globulin preparation III were precipitated over night with a saturated aqueous solution of picrolonic acid. After centrifugalizing the clear solution was shaken several times with iso-butyl alcohol. The alcohol gave no precipitate. The colorless solution was made up to 250 Cc. Even 3 Cc. of this did not protect against one L + dose of the toxin. Evidently the filtrate, *i. e.*, the centrifugalized solution after picrolonic acid contained no antitoxic units. This highly concentrated globulin corresponded in its reaction to picrolonic acid to preparation I which was of a lesser concentration.

The precipitate from picrolonic acid was shaken with NaCl (0.85 per cent.) and made into a suspension. This was shaken several times with iso-butyl alcohol to remove picrolonic acid. The isobutyl alcohol precipitated a gelatinous mass, which after centrifugalizing became colorless on further shaking with iso-butyl alcohol. This white gelatinous material was dissolved in NaCl (0.85 per cent.) by the addition of NaOH *n*/10 and the corresponding amount of *n*/10 HCl was then added. This colloidal solution was made up to 500 Cc. with NaCl (0.85 per cent.). This solution had antitoxic value, but did not correspond to the full number of units used. This may perhaps have been due to the long contact with iso-butyl alcohol.

The centrifugalized solution after iso-butyl alcohol precipitation was made up to 100 Cc. but even 3 Cc. did not protect against one L + dose, so that it contained few if any antitoxic units. Evidently iso-butyl alcohol precipitates antitoxin at least from this preparation.

Two Cc. of preparation III (8,000 antitoxic units) were precipitated with a saturated aqueous solution of picrolonic acid and the precipitate was shaken several times with NaCl (0.85 per cent.) centrifugalized, then the precipitate shaken again, then filtered through filter paper. The filtrate was then made up to 750 Cc. Even 3 Cc. did not protect from one L + dose of the toxin. The loss of activity may have been due to the filtering through filter paper, or to the preparation having stood several days, but it was thought, as there was picrolonic acid present, that this should preserve it.

10 Cc. globulin preparation I, diluted with an equal volume of

NaCl (0.85 per cent.), was precipitated with a saturated aqueous solution of uranium acetate c.p., then centrifugalized. The precipitate was dissolved in normal salt solution by the addition of a few drops of *n*/10 NaOH. The uranium was precipitated by Na_2HPO_4 and centrifugalized. This solution was diluted to 250 Cc., an arbitrary amount. It was found that one Cc. protected from one L+ dose. Less was not tried. This solution gave a precipitate with picrolonic acid, gold chloride, platinum chloride, copper acetate and alcohol. The filtrate after precipitation with Na_2HPO_4 and centrifugalizing was diluted to 250 Cc. Even 3 Cc. of this solution did not protect from one L+ dose. Uranium acetate precipitates antitoxin.

Globulin preparation I, diluted with an equal volume of normal NaCl, was cautiously precipitated with uranium acetate solution, then centrifugalized. The precipitate was treated with 15 Cc. NaCl (0.85 per cent.) and 15.6 Cc. *n*/10 NaOH. This formed an emulsion. The emulsion was precipitated with Na_2HPO_4, then precipitated with picrolonic acid. The precipitate was suspended in NaCl (0.85 per cent.) and shaken with isobutyl alcohol (Merck) to remove the picrolonic acid. The solution was diluted to 250 Cc. Even 3 Cc. did not protect from one L+ dose.

The iso-butyl alcohol gave a white precipitate which was made into a colloidal solution with 250 Cc. NaCl (0.85 per cent.). 2 Cc. of this solution protected against one L+ dose of the toxin.

Five Cc. of preparation II (2,250 units) were precipitated with platinum chloride (10 per cent.) aqueous solution. The precipitate was shaken with *n*/100 NaOH as well as with NaCl (0.85 per cent.) centrifugalized, neutralized and diluted to 350 Cc. Even 3 Cc. of this solution did not protect against one L+ dose of the toxin. A second sample was likewise precipitated with the same platinum chloride solution and the precipitate stirred with distilled water, then with NaCl (0.85 per cent.) and then centrifugalized. The washings from the precipitate and the centrifugalized solution from the platinum were added together and warmed on a bath to 65° C.; then, while warm, were saturated with H_2S and the gas boiled off in vacuo. In this case the platinum sulphide separated nicely giving practically a colorless solution. There is some difficulty in obtaining a colorless solution in every test. The solution was filtered through filter paper and diluted to 100 Cc. One Cc. protected against one L+ dose. After standing two days, one Cc. of

this solution was diluted to 22 Cc. Three Cc. of this dilution did not protect against one L + dose of the toxin.

The 100 Cc. solution gave no precipitate with uranium acetate c.p., gold chloride, mercuric chloride, or one half saturation with ammonium sulphate, but gave a slight precipitate with picrolonic acid. Picric acid gave no precipitate. This concentration gave no biuret reaction and no test for tryptophane with magnesium glyoxalate and sulphuric acid.

To see if the last dilution was inactive owing to deterioration, no extra-preservative having been added, the original 100 Cc. was tested 4 days after its preparation and 1 Cc. still protected against one L + dose of the toxin.

A similar precipitation was made with a freshly prepared solution of platinum chloride (10 per cent.) and the precipitate was washed with NaCl solution instead of with distilled water as in the preceding case. The washings and centrifugalized solution could not be freed from platinum by H_2S alone, even on adding an excess of platinum, but cleared with H_2S when 1 Cc. of HCl *n*/10 was added to the solution (111 Cc.). After dilution to 150 Cc. it was found that even 2 Cc. did not protect against one L + dose of the toxin.

Five Cc. of the same antitoxin was precipitated with a solution of platinum chloride made 2 days previously. The precipitate settling on centrifugalizing was washed with distilled water as in the first experiment and after passing H_2S became colorless save for a minute trace of golden color. This was diluted to 150 Cc. Even 1 Cc. protected against one L + dose of the toxin.

From these experiments it is evident that NaCl interferes with the precipitation of platinum unless acid is added.

There are several ways of interpreting the activity of the platinum filtrate; first, that it contained the antitoxin free from globulin or that the acidity, which resulted from passing H_2S, weakened the toxin, or that a trace of colloidal platinum sulphide remained in solution and weakened the toxin.

The acidity of the first platinum preparation corresponded to 0.3 Cc. *n*/10 HCl to each Cc. The second, in which much NaCl had been used and which was inactive, reacted for 0.55 Cc. *n*/10 HCl for each Cc. The third preparation which contained about as many antitoxic units as the first platinum preparation, reacted for 0.2 Cc. *n*/10 HCl to each Cc.

The L + dose of the toxin (0.42 Cc.) was treated with 0.42 *n*/10 HCl and let stand in the thermostat for ½ hour, then neutralized with 0.42 *n*/10 NaOH. This preparation killed a guinea pig in 24 hours, the same time as the untreated toxin. Evidently weakening of the toxin by acid was not the cause of the survival of the guinea pigs after injection of the toxin mixed with platinum filtrate from the globulin preparation.

To see if an excess of acidity was the cause of the inactivity of the second test, 1 Cc. of preparation II was mixed with 1 Cc. HCl *n*/10 placed in an incubator for ½ hour, then neutralized with NaOH. This was then diluted to 20 Cc., *i. e.*, to theoretically correspond to the dilution in the second platinum experiment. One Cc. of this solution protected against one L + dose of the toxin, thus showing that this amount of acid did not destroy the antitoxin.

As a control test, 7.5 Cc. of platinum chloride (10 per cent.) were diluted to 100 Cc. with distilled water and while warm were saturated with H_2S. On filtering this gave a solution perhaps darker in color than the preceding active platinum filtrate. This color was due to the presence of a trace of platinum sulphide. Injections were made of 1 Cc., 2 Cc., and 3 Cc. but these did not kill, or even sicken, the guinea pigs. Of this solution, 1 Cc. and 2 Cc. were each mixed with one L + dose of the toxin diluted as usual, and placed in the incubator for one half hour. Even 1 Cc. protected against one L + dose of the toxin, showing that the protection was due to the small amount of platinum present and that the antitoxin had not been freed. This action must presumably have been due to some catalytic action of the platinum as the concentration was presumably too weak to precipitate any of the toxin. These results may suggest a therapeutic use for platinum compounds.

From our review of the literature and from our own work at present we find no chemical proof that a separation of antitoxin and globulin can be made, although Banzhaf's work and that of Hurwitz and Meyer might suggest it.

Note.—Several of the guinea pigs on which the platinum experiments were made developed abscesses.

ASSAY PROCESSES OF THE U. S. P. IX.

By Philip Asher, Ph.G., M.D.

(*Concluded from page 121.*)

IODOMETRY.

Iodometric methods are followed in quite a number of U. S. P. assays. These may be divided into two classes. First, those in which direct addition of *n*/10 iodine forms with the substance under examination a definite compound, and the completion of the reaction is shown by the production of a blue color with starch. Secondly, those in which either an excess of iodine is added, or iodine is liberated by the addition of potassium iodide, and the liberated iodine titrated with sodium thiosulphate.

To class one belong the assays of tartar emetic, arsenic trioxide and sodium thiosulphate.

Antimony and potassium tartrate. .5 Gm. of the salt is dissolved in 30 mils of water, to which are added 25 mils cold saturated sodium bicarbonate solution, and starch as an indicator. This is immediately titrated with *n*/10 iodine.

$$2KSbO(C_4H_4O_6) + I_4 + 8NaHCO_3 = 2NaSbO_3 + 4NaI + 2KNaC_4H_4O_6 + 8CO_2 + 5H_2O.$$

Tartar Emetic — Iodine — Sodium Bicarbonate — Sodium Meta Antimonate — Sodium Iodine — Potassium Sodium Tartrate

Arsenic trioxide. .2 Gm. arsenic oxide accurately weighed is dissolved in 20 mils of hot water by the gradual addition of NaOH T. S. This is neutralized with dilute sulphuric acid. To the cooled solution, sodium bicarbonate is added and titrated with iodine.

$$As_2O_3 + H_2O + 4I + 4NaOH = 2H_3AsO_4 + 4NaI.$$

Arsenic Trioxide — Iodine — Sodium Hydroxide — Arsenic Acid — Sodium Iodide

In the second class are the assays of the ferric salts, crude calcium sulphide, chromium trioxide, iodine, acetone, sodium bisulphite and arsenate, phenols, thymol iodide, mercury salicylate, and mercurous iodide and chloride.

The assay of crude calcium sulphide introduces a new method. To the salt, water is added and ammonium chloride solution, and allowed to stand for a short time. Then cadmium chloride is added, and after agitating, some acetic acid is added and the whole heated for 15 minutes. The supernatant liquid is decanted through a filter, and the precipitated cadmium sulphide agitated with acetic acid, and the precipitate is washed with acetic acid. The precipitate is returned to the flask, iodine solution added, and HCl. The flask is stoppered, and the excess titrated with sodium thiosulphate.

Acetone. In this assay the acetone is treated with KOH and iodine solution, and HCl, and titrated with sodium thiosulphate. The iodine converts the acetone into tri-iodoacetone and hydriodic acid:

$$\underset{\text{Acetone}}{CH_3COCH_3} + \underset{\text{Iodine}}{I_6} = \underset{\text{Tri-iodo-acetone}}{CI_3COCH_3} + \underset{\text{Hydriodic Acid}}{3HI}.$$

The tri-iodoacetone is then acted upon by the KOH, being converted into iodoform and potassium acetate, and the hydriodic acid neutralized by the KOH:

$$\underset{\substack{\text{Tri-iodo-}\\\text{acetone}}}{CI_3COCH_3} + \underset{\substack{\text{Hydriodic}\\\text{Acid}}}{3HI} + \underset{\substack{\text{Potassium}\\\text{Hydroxide}}}{4KOH}$$

$$= \underset{\text{Iodoform}}{CHI_3} + \underset{\substack{\text{Potassium}\\\text{Iodide}}}{3KI} + H_2O + \underset{\substack{\text{Potassium}\\\text{acetate}}}{CH_3COOK}.$$

The excess of iodine forms with the alkali potassium iodide and iodate, and these are decomposed by the HCl into iodine. The excess of iodine used is determined by sodium thiosulphate.

Phenols are assayed by adding to them $n/10$ to bromine solution and HCl, allowing to stand 15 minutes, and quickly adding potassium iodide, care being taken to avoid loss of bromine or iodine by keeping the flask tightly stoppered. After standing for some time, 1 mil chloroform is added, shaken, and excess of iodine titrated. This assay takes place in three stages. In the first, the bromine combines with the phenol, forming tribromphenol:

$$\underset{\text{Phenol}}{C_6H_5OH} + \underset{\text{Bromine}}{Br_6} = \underset{\text{Tribromphenol}}{C_6H_2Br_3OH} + \underset{\substack{\text{Hydrobromic}\\\text{Acid}}}{3HBr}.$$

The next step is the liberation of iodine by the free bromine:

$$\underset{\text{Potassium Iodide}}{2KI} + \underset{\text{Bromine}}{Br_2} = \underset{\text{Potassium Bromide}}{2KBr} + \underset{\text{Iodine}}{I_2}.$$

In the last stage, the decolorization of the iodine by the sodium thiosulphate:

$$\underset{\text{Sodium Thiosulphate}}{2Na_2S_2O_3} + \underset{\text{Iodine}}{I_2} = \underset{\text{Sodium Iodide}}{2NaI} + \underset{\text{Sodium Tetrathionate}}{Na_2S_4O_6}.$$

Thymol iodide, $C_{20}H_{24}O_2I_2$. The assay of this compound differs from general methods. The thymol iodide is mixed with sodium carbonate and heated in a crucible until carbonized. The residue is extracted with water and washed on a filter until an opalescence is no longer formed with silver nitrate. The solution is heated and treated with potassium permanganate until a faint pink color remains. Sufficient alcohol is added to remove the pink tint, and the solution cooled. Water is added to make 200 mils. It is mixed and filtered. To 100 mils of the filtrate, potassium iodide is added, and an excess of sulphuric acid, and the liberated iodine is titrated. The principles underlying the above are the following: The iodine of the thymol is converted into sodium iodide when heated with the sodium carbonate. The mixture is converted into sodium iodate by the potassium permanganate. The action of the sulphuric upon the sodium iodate and potassium iodide results in the formation of hydriodic acid and iodic acid, and these reacting upon each other liberate iodine.

Sodium arsenate. A weighed amount of the salt is dissolved in water, and heated to 80° C. HCl is added and potassium iodide. After standing for some time, the liberated iodine is titrated. In this assay the following reactions take place: The HCl decomposes the salts, forming their respective acids:

$$\underset{\text{Potassium Iodide}}{2KI} + \underset{\text{Sodium Arsenate}}{Na_2HAsO_4} + \underset{\text{Hydrochloric Acid}}{4HCl} = \underset{\text{Potassium Chloride}}{2KCl} + \underset{\text{Sodium Chloride}}{2NaCl} + \underset{\text{Hydriodic Acid}}{2HI} + \underset{\text{Arsenic Acid}}{H_3AsO_4}.$$

The acids then react upon each other:

$$\underset{\text{Hydriodic Acid}}{2HI} + \underset{\text{Arsenic Acid}}{H_3AsO_4} = \underset{\text{Iodine}}{I_2} + \underset{\text{Arsenous Acid}}{H_3AsO_3} + H_2O.$$

In the assays of mercurous chloride and iodide, an interesting phase is introduced. The salt is mixed in a flask with water, iodine solution is added, and potassium iodide, and the mixture allowed to stand, with occasional agitation, until complete solution has taken place, and the excess of iodine is titrated. With mercurous chloride the potassium iodide converts it into mercurous iodide, and it is subsequently changed into mercuric iodide by the oxidation of the iodine:

$$\underset{\text{Mercurous Chloride}}{HgCl} + \underset{\text{Potassium Iodide}}{KI} = \underset{\text{Mercurous Iodide}}{HgI} + \underset{\text{Potassium Chloride}}{KCl.}$$

Then

$$\underset{\text{Mercurous Iodide}}{HgI} + \underset{\text{Iodine}}{I} = \underset{\text{Mercuric Iodide}}{HgI_2},$$

which is soluble in potassium iodide.

In the assay of mercury salicylate, the above method is also followed, but a preliminary reduction to mercurous chloride is made. The salt is first treated with sulphuric and nitric acids and digested on a water bath until dissolved. This produces mercuric sulphate and nitration products of salicylic acid. The solution is diluted with water and hydrogen peroxide added to oxidize anything that may have a tendency to reduce the mercury to a metallic condition. The solution is then treated with hypophosphorous acid, followed by sodium chloride. The hypophosphorous acid reduces the salt to the mercurous state, and the sodium chloride then converts it into mercurous chloride. The precipitate is thoroughly washed, and the precipitate and filter are returned to the flask, and the method is then followed as under mercuric chloride.

POTASSIUM PERMANGANATE METHOD.

There are quite a number of the official compounds, the strengths of which are ascertained by titration with *n*/10 potassium permanganate. Some of these are by direct titration or by an excess of the permanganate, and titrating such excess with oxalic acid. Others are first converted into oxalate by the addition of an excess of oxalic acid, and this excess is subsequently titrated with permanganate, or by conversion of soluble salts into oxalates by means of ammonium oxalate. To the first class belong the ferric salts, hydrogen peroxide, sodium nitrite and perborate. In the latter class

are calcium carbonate and oxide, solution lead subacetate, lead acetate and oxide and manganese dioxide. For example, calcium carbonate is first converted into the chloride by HCl. To this, oxalic acid is added, and sufficient ammonia water to make alkaline. After standing over night, the solution is filtered, washed, acidified with sulphuric acid, and the excess of oxalic acid titrated with permanganate.

In the assay of potassium chlorate several new features are introduced. .1 Gm. of potassium chlorate is dissolved in 10 mils of water, to which is added 25 mils acidulated ferrous sulphate T.S. The solution is placed in a flask with a valve stopper, made by cutting a longitudinal strip 15 Mm. long in a rubber tube and closing the end with a glass rod. This allows for the escape of gases without permitting the air to return to the flask. The solution is boiled 10 minutes, cooled, and 10 mils manganese sulphate solution added, and the excess of ferrous sulphate titrated with potassium permanganate. A parallel is run without potassium chlorate. The result of the former is subtracted from that of the latter. The reactions involved are as follows:

$$\underset{\text{Potassium Chlorate}}{KClO_3} + 6\underset{\text{Ferrous Sulphate}}{(FeSO_4\cdot 7H_2O)} + \underset{\text{Sulphuric Acid}}{3H_2SO_4} = \underset{\text{Ferric Sulphate}}{3Fe_2SO_4)_3} + \underset{\text{Potassium Chloride}}{KCl} + 45H_2O.$$

$$\underset{\text{Potassium Permanganate}}{2KMnO_4} + 10\underset{\text{Ferrous Sulphate}}{(FeSO_4\cdot 7H_2O)} + \underset{\text{Sulphuric Acid}}{8H_2SO_4} = \underset{\text{Ferric Sulphate}}{5Fe_2(SO_4)_3} + \underset{\text{Potassium Sulphate}}{K_2SO_4} + \underset{\text{Manganese Sulphate}}{2MnSO_4} + 8H_2O.$$

The assay of sodium peroxide presents nothing of special moment, except the fact that when it is dissolved in water, it decomposes into H_2O_2 and sodium metaborate. The assay is conducted by adding to the weighed salt dissolved in water, sulphuric acid, and titrating with potassium permanganate.

$$\underset{\text{Sodium Perborate}}{NaBO_3\cdot 4H_2O} + H_2O = \underset{\text{Hydrogen Dioxide}}{H_2O_2} + \underset{\text{Sodium Metaborate}}{NaBO_2} + 4H_2O.$$

$$\underset{\text{Hydrogen Dioxide}}{5H_2O_2} + \underset{\text{Potassium Permanganate}}{2KMnO_4} + \underset{\text{Sulphuric Acid}}{3H_2SO_4}$$

$$= 2O_5 + \underset{\text{Potassium Sulphate}}{K_2SO_4} + \underset{\text{Manganese Sulphate}}{MnSO_4} + 8H_2O.$$

There is only one assay in which potassium dichromate is used, saccharated ferrous carbonate. The salt is dissolved in diluted sulphuric acid, and immediately titrated with potassium dichromate, potassium ferricyanide being used as an indicator:

$$\underset{\text{Ferrous Sulphate}}{6FeSO_4} + \underset{\text{Potassium Dichromate}}{K_2Cr_2O_7} + \underset{\text{Sulphuric Acid}}{7H_2SO_4}$$

$$= \underset{\text{Ferric Sulphate}}{3Fe_2(SO_4)_3} + \underset{\text{Potassium Sulphate}}{K_2SO_4} + \underset{\text{Chromium Sulphate}}{Cr_2(SO_4)_3} + 7H_2O.$$

There are but few of the compounds and preparations of the U. S. P. in which potassium sulphocyanide is used: silver nitrate, solution arsenic and mercury iodide, mass of mercury, mercury and chalk and mercuric oxide.

In the assay of mercuric oxide the mercuric compound is treated with nitric acid, whereby it is converted into mercuric nitrate. This is diluted with water, ferric alum added as an indicator, and titrated with sulphocyanide of potassium. In this process, the mercury is precipitated by the sulphocyanide as the insoluble mercuric sulphocyanide, the completion of the reaction is shown by the formation of a yellowish-red color.

The mercury of solution of arsenic and mercury iodide, is determined by adding to the solution KOH and then formaldehyde, by which the mercury is reduced to the metallic condition. The mercury is washed by decantation, and dissolved by nitric acid. The same process is followed as under the oxide.

In determining mass of mercury, the substance is treated with sulphuric and nitric acids whereby mercuric nitrate is formed, and the organic matter is destroyed. Potassium permanganate is also added until a pink tint is produced, making certain that no organic matter remains, and the color discharged by oxalic acid T.S. The sulphocyanide method is then followed.

GASOMETRIC METHODS.

But three substances of the Pharmacopœia are assayed gasometrically: oxygen, amyl nitrite, and spirits ethyl nitrite. The determination of the two latter is carried out in a nitrometer, supplied

with control tubes. The nitrometer is completely filled with saturated salt solution, care being taken that no air is present. When ready the control tube is placed at a low level. The substance to be assayed is first prepared by adding to it potassium bicarbonate, to remove any acid that may be present. The quantity of material to be assayed is added to the nitrometer, followed by potassium iodide and sulphuric acid. The whole is shaken, and after the reaction has ceased, the equilibrium tube is raised to the level of the liquid in the nitrometer and the reading taken. Temperature and barometric pressure should also be considered.

$$\underset{\text{Ethyl Nitrite}}{C_2H_5NO_2} + \underset{\text{Potassium Iodide}}{KI} + \underset{\text{Sulphuric Acid}}{H_2SO_4} = \underset{\text{Alcohol}}{C_2H_5OH} + \underset{\text{Potassium Bisulphate}}{KHSO_4} + \underset{\text{Iodine}}{I} + \underset{\text{Nitric Oxide}}{NO.}$$

The purity of oxygen is determined by placing fifty mils of it into an accurately calibrated tube, with 10 mils of alkaline pyrogallol solution; not less than 95 per cent. by volume should be absorbed.

MISCELLANEOUS ASSAYS.

The refractometer is directed to be used in determining the purity of several of the volatile oils.

The polariscope is used principally in the testing of volatile oils. Its use is also directed in the assay of spirits and liniment of camphor.

The assay is conducted by taking the mean of 4 polariscopic readings of the spirit in a 200 Mm. tube. Correction to be made for temperature. 60 mils of the spirits is also evaporated on a water bath, and when the camphor begins to solidify, it is stirred until dry. The camphor is then placed on a watch crystal, covered with an inverted funnel, and heated by a Bunsen burner to sublime the camphor. 2.5 Gm. of the sublimate are dissolved in sufficient 95 per cent. alcohol to make exactly 25 mils, and the mean of four polariscopic readings are taken. The minutes of rotation of the spirits divided by the minutes of rotation of control, multiplied by 10 gives the grams of camphor in a hundred mils of the spirit.

SAPONIFICATION.

Saponification methods are used in the U. S. P. in assaying fats, resins, wax, and volatile oils. The saponification value indicates the number of Mgs. of KOH required to saponify one gram of

the oil. The acid number for resins implies the number of Mgs. KOH required to neutralize one gram of the resin.

One compound of the U. S. P., liquid petrolatum, contains a viscosity test. It is conducted by placing a mark at 2 Cm. below the bulb of a 50 mil pipette, filling with distilled water to the upper level, and noting the time in seconds required for the level of the water to reach the lower mark. The liquid to be tested, is then drawn to the upper mark of the pipette, and the time in seconds it requires to reach the lower level, divided by the number of seconds taken by the water, indicates its viscosity.

Under each drug is given the percentage of the limit of ash it should contain.

Some of the zinc and mercury compounds are tested electrolytically.

Amylolytic and proteolytic methods are also included among the U. S. P. assay methods; the former for pancreatin, diastase and malt; the latter for pepsin.

In addition to the chemical methods of assay, several of the drugs are directed to be assayed biologically: aconite, digitalis, strophanthus and squills. The biological method is also used in connection with biological substances now included in the Pharmacopœia: suprarenal gland, dried hypophysis, serums, etc.

ALKALOIDAL ASSAYS.

The active constituents of a large number of the vegetable drugs reside in alkaloids. Time will not permit of detailing these methods. With but one or two exceptions the alkaloids are determined volumetrically. The drug is macerated with a mixture of one volume of chloroform and two of ether. After standing a short time ammonia water is added, and shaken vigorously every ten minutes for two hours. Some water is added, and the whole allowed to settle. The liquid is decanted, and an aliquot part filtered through cotton into a separator. The alkaloids are extracted by shaking out with weak sulphuric acid. The acid washings are made alkaline with ammonia water and are extracted by repeated shakings with chloroform. The chloroform solution is evaporated and dissolved in an excess of *n*/10 sulphuric acid, and the excess of acid used is titrated with *n*/50 alkali, cochineal being used as an indicator. From the number of mils of acid consumed, the per cent. of alkali is determined by multiplying by its coefficient, and dividing by the

amount of drug used. Preparations containing alkaloids are similarly treated. They are first evaporated to a small bulk and then the extraction is carried out as above outlined.

Cantharides.

Cantharides is assayed by macerating 15 Gm. of the drug with 150 mils of a mixture of 2 volumes of benzene and 1 volume of benzine to which has been added 2 mils of HCl. It is allowed to stand 10 hours then warmed to 40° C. and maintained at this temperature and frequently agitated for three hours and cooled. 100 mils are decanted and evaporated to about 5 mils. To the evaporated solution are added 5 mils of chloroform and set aside. After the solvent has evaporated an equal volume of 10 mils of dehydrated alcohol and benzine saturated with cantharidin, are added to the crystals and allowed to stand 15 minutes. The liquid is decanted through a pellet of cotton and the crystals washed with successive portions of the saturated cantharidin solution to remove fat and coloring matter and the washings passed through the cotton. The cotton is then washed with warm chloroform and the washings added to the beaker containing the crystals and the solvent evaporated by a blast of air and the crystals are dried at 60° C. and weighed. The official requirement is that cantharides should contain not less than .6 per cent. of cantharidin.

Assays Needed.

The revisers of the U. S. P. have performed an excellent piece of work, but the assays for a large number of preparations are just as essential as the assays of the substances from which they are made, and these should have been included. While it is true that the articles entering into the composition of these preparations may be up to the official requirements, what assurance is there that the preparations made therefrom are of standard strength? While these standards will hold in check the manufacturers of chemicals and the jobbers selling the official drugs, etc., there is nothing in the official requirements to hold a pharmacist who may attempt to make his preparations deficient in strength.

The following preparations should have included an assay method: Emulsions of asafœtida and cod liver oil; especially a test for the former to show that it had not been prepared from the tincture. The glycerites, boroglycerite and tannin; the spirits should

have an assay method, particularly to show the amount of the volatile oils present. Also the following: Solution cresol compound, liniments of Belladonna and chloroform; ointments of belladonna, tannic acid, boracic acid, mercury, ammoniated mercury, mercury nitrate, iodine, iodoform, stramonium, sulphur and zinc oxide; mercury oleate; pulv. ipecac and opium and cerate cantharides.

TECHNICAL EDUCATION AND METRICS.

By H. V. Arny, Ph.D.

It is a great privilege to bring to the National Association of Manufacturers of Medicinal Products the greetings of the American Conference of Pharmaceutical Faculties.

This organization, over which I had the honor of presiding last year, consisting of *42* of the best schools of pharmacy in this country, was organized in 1900 for the purpose of promoting the cause of pharmaceutical education. It has performed its work well in the past. It has a greater mission for the future, and this mission is of personal interest to every member of the Association of Manufacturers.

In the past much of our work has been the very important task of fitting young men for retail pharmacy in a course of instruction covering two years. This always has been and always will be a vital function of the College of Pharmacy. Of scarcely less importance, however, is the training of young men for technical positions in your manufacturing plants in a course covering four years. Are we, the colleges of the conferences, doing our duty to you in this direction? If we are not, you practical and energetic business men should coöperate with us in making courses in technical pharmacy what they should be.

There are three ways in which the pharmaceutical industries should coöperate with the colleges in making these four year technical courses a success.

Two of the three ways are already in vogue to a certain extent. These are: (*a*) Permitting experts from your plants to give one or more special lectures in the courses on chemical technology given in our colleges; (*b*) permitting our technical students to visit your plants.

The third and most important type of coöperation is one, which, as explained at some length by the writer in a special article on the subject, is based on the establishment of industrial fellowships in pharmacy along the lines instituted by the late Robert Kennedy Duncan and now seen in its fulness at the Mellon Institute at Pittsburgh.

Reduced to its simplest terms, the idea is this: You manufacturers turn to the colleges for young men to *break in* for service your plants; you manufacturers have problems requiring research that some of us teachers might aid you solve. Why would it not be feasible to turn such problems over to those of our colleges of pharmacy providing suitable technical courses? Let the proposition take the shape of an industrial fellowship; that is, let the teacher select some likely graduate to carry out the laboratory work under his supervision. The expense of the research to be borne by the interested manufacturer. Such an Industrial Fellowship would perform the triple service of (*a*) encouraging research, (*b*) trying out for you a likely employee without wasting the time of your own force in the experiment, (*c*) training the aforesaid likely employee in the very lines in that he would be of most service to you. If Duncan's industrial fellowship idea has proven of distinct value in the chemical industry, there is no reason why the same idea could not be applied with great success in a true coöperation between the Association of Manufacturers and the Colleges.

I am present with you this morning not merely to convey the greetings of the Conferences of Faculties, but also as a representative of the newly organized AMERICAN METRIC ASSOCIATION. This Association was formed on December 27, last, and the best proof of your interest was shown in the appointment by your executive board of Mr. George Simon to represent the Association of Manufacturers on that occasion. Nor do I need to take much of your time in telling you the advantages of the metric system. From the days when Dr. Squibb marketed his pharmaceutical products in metric packages, to the present time, when many of you prepare metric price lists for export business, manufacturing pharmacy has been farsighted enough to see that the sooner we become a metric country the better.

At this time I wish to thank the representatives of the Association of Manufacturers in the Drug Trade Conference for their coöperation in securing the endorsement of the new A. M. A. by that body.

I wish also to mention how splendidly pharmacy was represented at the organization meeting of our metric association: a representation so appreciated by the others that two druggists—Dr. Wm. J. Schieffelin and the speaker—were placed on the list of those selected to conduct the affairs of the Association. The list of officers should be mentioned to show the type of business men now interested in metrics. They are: *President, George F. Kunz* of New York, gem expert and chairman of the metric committee of the American Institute of Mining Engineers. *First Vice-President, Wm. Jay Schieffelin,* of New York, wholesale druggist and member of the metric committee of the N. W. D. A. *Second Vice-President, Emil P. Albrecht,* secretary of the Philadelphia Bourse. *Third Vice-President, Orrin E. Stanley,* of Portland, Oregon, civil engineer and secretary of the Society for the Promotion of the Metric System. *Secretary, Howard Richards, Jr.,* of New York, electrical engineer and founder of the Metric Association of China. *Treasurer, Arthur P. Williams,* of New York, wholesale grocer and chairman of the trade committee of the National Wholesale Grocers Association. *Executive Committee, H. V. Arny,* New York, chemist; *F. R. Drake,* Easton, Pa., wholesale grocer; *S. L. Stratton,* Bureau of Standards, Washington, D. C.; *W. P. Wilson,* director Phil. Commercial Museum; *A. E. Kennelly,* Cambridge, Mass., electrical engineer. At present, the only paid official is the assistant secretary, a young man, doing our stenographic work. But we hope to soon be in a position to employ an executive secretary for field work.

Noteworthy is the interest of the Wholesale Grocers and the Canners in the Metric Association. Their interest is not purely academic as their use of metric quantities on the "net weight" statements on their labels clearly show.

As to membership the Association provides three classes:

1. *Individual members,* with dues of not less than $2 a year.
2. *Firms,* with dues of not less than $5 a year.
3. *Associations,* with dues of not less than $10 a year.

Each member of the Association is permitted five (5) delegates at our annual convention.

I dare to express the hope that each person in this room will become an *individual member,* and that many of you will see that your firms take out membership also.

As to the Association of Manufacturers itself, the fact that this body had representation at the organization meeting of the Metric

Association leads the officers of that body to count upon you as a part of us; and I hope that at this meeting your organization may see fit to become a member of the Metric Association. We want you with us. We have placed organization membership at a low figure ($10 per annum) to show that we primarily wish the moral support of friendly organizations. In passing, I might point out that some organizations—The National Wholesale Grocers Association, and the Philadelphia Bourse, for instance, have gone further than mere membership, each of these bodies giving us a $50 donation.

And now I think I hear some of you saying: "But what's behind the whole thing?" In answer I will say that the organization of the METRIC ASSOCIATION is a tangible expression of the opinion of practical men—engineers, chemists, grocers, druggists, merchants and a sprinkling of those "theoretical fellers," the teachers—that now is *the* psychological moment to throw over the archaic standards with which we have been wrestling all these years and to turn to the international language of commerce, *The Metric System.* We, who appreciate the value of the metric system, must educate our business friends, who do not yet understand its time-saving and its trade-getting qualities; and when these qualities are understood the transition from the old units to the new will be easily accomplished.

The officers of the Metric Association are a unit in the opinion that metric education must precede metric legislation. But they also believe that their metric propaganda plus the international calls of to-day will surely result in bringing all practical Americans to a realization of the fact that it is high time for this country of ours to throw off the shackles of an Elizabethan set of standards and to add our 110,000,000 people to the 437,000,000 already using the metric system.

PUBLICATION OF INFORMATION ON DETAINED IMPORTS OF FOOD AND DRUGS AT PORTS OF ENTRY.

The Bureau of Chemistry, Department of Agriculture, gave a public hearing in the building of the Bureau of Chemistry at Washington, D. C., on Tuesday, March 20, 1917, at 10 A.M., to consider the question of publishing data on the detention of food and drugs offered for import at ports of entry. Dr. Carl L. Alsberg presided.

What the department wished particularly to ascertain was the opinion of the trade upon the desirability of publishing such information, and the form of such publication, and also, as to whether or not such publication would injure a consignee importing goods from abroad who has had no opportunity of inspecting the same prior to their arrival and detention at ports of entry.

Representatives were present from the National Wholesale Druggists Association, the Philadelphia Drug Exchange, the Drug Trade Section of the New York Board of Trade and Transportation, the National Association of Retail Druggists, and a number of national food organizations. Briefs from various trade bodies were filed, also.

After an extended discussion of the subject from many angles, the consensus of opinion expressed seemed to be:

(1) That it was undesirable to publish information relative to detained shipments unless the shipments gave evidence of intentional and wilful violations of the law, when the facts should be made public, (2) that the Bureau of Chemistry should coöperate with a Committee on Standards to be named by the various national food and drug interests with the view of framing tentative standards and tests for imported food and drugs. It was shown that there was precedent for such coöperation in Government work, *e. g.*, Seed Department of Bureau of Plant Industry, (3) that all the methods and tests used by the Bureau of Chemistry should be made public so that importers could know in advance of ordering goods what standards to specify, (4) that the sampling and methods of examination of drugs should be made uniform at all the ports of entry, (5) that the Bureau of Chemistry coöperate with the food and drug trade in securing an amendment of the Federal Food and Drugs Act giving the importers the right of appeal to a court, preferably the Board of General Appraisers.

If practicable standards are framed and proper publicity is given them so that the foreign exporter and the domestic importer shall have full knowledge of the same, it was felt that better conditions would result and that there would be little or no necessity for publishing information relative to detained shipments.

Dr. Carl Alsberg, chief of the Bureau of Chemistry, would not, of course, commit himself as to the attitude of his department on these suggestions, but stated that he would give them careful consideration, and that he wanted the assistance and coöperation of the trade

represented by the food and drug industries. He will decide later what can be done.

J. W. ENGLAND.

POSSIBILITY OF THE COMMERCIAL PRODUCTION OF LEMON-GRASS OIL IN THE UNITED STATES.[1]

BY S. C. HOOD, SCIENTIFIC ASSISTANT, DRUG-PLANT AND POISONOUS-PLANT INVESTIGATIONS.

Lemon-grass oil is the volatile oil distilled from the plant known botanically as *Cymbopogon citratus* DC. and commonly called lemon grass. It is lemon yellow to brownish in color, with a strong odor resembling that of the lemon verbena, and for many years has occupied a prominent place in the perfume industry. The value of this oil depends almost entirely upon its content of citral, which is used in the manufacture of ionone, or artificial violet. Considerable use is also found for the oil in the soap industry.

The principal regions where lemon-grass oil is produced are the Travancore Province and Madras Presidency of India and the island of Ceylon. Small quantities are regularly produced in other parts of the East Indies, and from time to time in many other parts of the world.

Exact figures are not available regarding the consumption of lemon-grass oil in the United States, but estimates place it at about 100,000 pounds annually.

For the past eight years the Bureau of Plant Industry has been conducting experiments in the growing of lemon grass in central Florida, and during the course of the experiments field tests have been made with 13 varieties secured from eight different parts of the world.

SOIL AND CLIMATIC REQUIREMENTS OF LEMON GRASS.

The best results with lemon grass have been obtained on well-drained sandy loam, but this plant also does well on light sand, such as the high pine lands of the Florida peninsula. Newly cleared sandy pine land without the previous application of lime has also given good results. Soil which is poorly drained or underlain by hardpan

[1] Reprinted from Bulletin No. 442, Bureau of Plant Industry, U. S. Department of Agriculture.

within 3 feet of the surface should not be planted to lemon grass. Field tests have not been made on heavy clay lands, but the successful cultivation of the crop on that type of soil is regarded as doubtful.

The climatic requirements of lemon grass are subtropical. A winter temperature of 28° F. has killed the plants to the ground, while 24° has killed the roots. However, the crop may be planted with safety where the temperature does not fall below 25° F., and under certain conditions even a slightly lower temperature may not cause serious damage.

PROPAGATION.

Lemon grass does not produce seed in this country, although occasionally an abortive flower spike may be found on old, neglected plants. Propagation, therefore, is effected by division of the clumps. From each clump 25 to 50 divisions may be separated easily by tearing them off from the base of the mature plant. This should be accomplished by a sidewise pull, so that a few root fibers will be retained on each division. In case the old plants are to remain in their places the required number of divisions can be secured by pulling them off from the outer edge of the old clump. With a little practice these may be removed without loss of root fibers.

Before planting, the tops of the divisions should be cut back to about 3 inches. The plants should be set in the early spring in rows 3 feet apart and about 18 inches apart in the row. This work should be done just after a rain or at a time when the soil is sufficiently moist not to require artificial watering.

FERTILIZERS AND CULTIVATION.

The results obtained from experimental fertilizer plats seem to indicate that on the sandy Florida soils rather more potash is required by lemon grass than by most grasses. Analysis shows a considerable variation in the percentage of nitrogen, phosphoric acid, and potash present in the plants of the different varieties tested. The results secured with one variety, which may be taken as a type, show that 5 tons of lemon grass contain 20.32 pounds of nitrogen, 33.20 pounds of potash, and 18.75 pounds of phosphoric acid. In the fertilizer tests a better growth was secured when the potash was applied in the form of the sulphate, and the results were more satisfactory when part of the nitrogen was applied in organic form. In the tests which have been made a fertilizer having 4 per cent.

nitrogen, 5 per cent. potash, and 8 per cent. phosphoric acid, applied at the rate of 600 pounds to the acre, has given the best results with the least cost. On soils of higher fertility a smaller quantity could be used. Although the use of larger quantities of fertilizers will give a heavier growth, it is by no means certain that the additional cost will be met by the increase in the crop.

As soon as the plants have become well established in the field the fertilizer should be given as a side application and well worked in at the first cultivation. Cultivation should be frequent throughout the spring, to conserve the soil moisture, and throughout the summer all weeds should be kept down, as a few ill-smelling weeds in the crop at harvest time will greatly injure the odor of the oil. After the first year, only slight cultivation is needed, since after it is well established lemon grass tends to retard weed growth.

HARVESTING.

The first cutting should be made four or five months after planting, at which time the plants should be from 2½ to 3 feet high and the bunches from 8 to 10 inches in diameter. Although the plants will continue to grow throughout the summer, it has been found that after a certain size has been reached the increase in weight is less rapid; hence, it is more profitable to harvest the crop at the time stated and allow a new growth to develop. In the early fall of the first year a second cutting can be secured. After the first year the growth in the spring is more rapid and three harvests a year can be obtained. Harvesting can be accomplished by the use of a mowing machine so adjusted as to cut the plants about 8 inches above the ground. The cut material can be raked up with a horse-rake run crosswise of the rows.

In order to determine the proper stage and height at which the plants should be cut to produce the best yield and quality of oil, a number of tests were made, covering several years. In 1908 the plants were cut when they were 2 feet high. They were then tied in bundles, the bundles cut into three 8-inch lengths, and each portion distilled separately. The yield of oil obtained from each portion, together with the citral content of the oils, is shown in Table I.

From these results, which are borne out by additional data obtained in succeeding years, the conclusion is evident that close cutting will not be profitable, because of the low oil content in the lower portion of the plant.

TABLE I.

Yield and Citral Content of Lemon-grass Oils Distilled from Plants 2 Feet High.

	Yield of Oil.	Citral Content of the Oil.[2]
Upper third	0.46	70
Middle third	.24	78
Lowest third	.10	82

For the purpose of determining whether the hauling cost could be reduced by drying the plants before taking them to the still, the following test was made: A quantity of fresh plants was collected, well mixed, and divided into three portions. The first portion was distilled green, the second portion was exposed to the sun for several hours until the blades were nearly dry, and the third portion was dried in a loft for several hours at 110° F. The two dried portions were then distilled separately and the yield of oil calculated on the original green weight of the material. The results secured, together with the citral content of the oils, are given in Table II.

These results show that there was considerable loss of oil by

TABLE II.

Yield and Citral Content of Lemon-grass Oils Distilled from Green and from Dried Plants.

Condition of Material.	Weight of Material (Green).	Weight of Material (Dried).	Yield of Oil (Based on Green Weight of Material).	Citral Content of the Oil.
	Pounds.	*Pounds.*	*Per Cent.*	*Per Cent.*
Fresh	78.1	...	0.37	78
Sun dried	93.1	58.3	.31	78
Artificially dried	100.3	62.7	.32	79

drying the plants. In the case of the sun-dried plants the loss on a 4-ton crop would be 4.8 pounds of oil, or, at the prices prevailing for 1915, a loss of $3.84, which would more than pay for the extra hauling charge. Drying the plants seems to have no effect on the citral content of the oil, but on storing it was found that the solubility of the oil in alcohol diminished more rapidly in the oils from the dried material.

[2] The citral content throughout all the experiments was determined by the sodium-sulphite method.

DISTILLATION.

The apparatus required for the distillation of lemon-grass oil does not differ from that in general use for the distillation of other volatile oils. Before distilling the plants it has been found advisable to run them through a fodder cutter, in order to permit closer packing in the retort. From the data at hand it is estimated that if the plants are cut into 2-inch lengths a retort will hold 100 pounds of material for every 6 cubic feet of space, but if the plants are put in whole the quantity which the retort can hold will be somewhat less. The closer packing, however, in no way facilitates distillation.

In a retort having a capacity of 30 cubic feet a charge of 3,000 pounds can be distilled in 2 to 2½ hours by the steam which may be readily generated in a small farm boiler, and by the use of a larger volume of steam the time can be much reduced.

In this connection it is interesting to note that distillation under 20 pounds pressure in the retort increased the yield of oil, but gave an oil of very dark color and with lower citral content.

After the oil has been distilled it should be freed from water so far as possible in a separatory funnel, then dried by shaking with anhydrous calcium chlorid, and filtered. It should be stored in well-filled air-tight containers in as cold a place as possible until ready to be shipped to market. The shipping can be done in new and clean tin cans without injury to the product.

In order to determine whether any appreciable quantity of oil would be lost by discarding the distilled water coming over with the oil, a series of tests was made in 1915. The water from a number of charges of several pounds each was retained and each lot separately redistilled. In the apparatus used in the experiments about 1 gallon of water was secured for each 22 pounds of herb in the charge. The average of the results secured by the redistillation of this water showed that 1.2 gram of oil was dissolved in each gallon of water, a quantity too small to make its recovery profitable. Examination of this recovered oil showed its characteristics to be practically identical with the oil distilled directly from the herb.

VARIETIES.

During the many years that lemon grass has been cultivated a great variety of forms of the plant has been developed. Some years ago an attempt was made to divide the old species into two separate species, basing the descriptions partially on the character of the oil

secured from the two sorts. In the essential-oil trade it long has been recognized that there is a wide difference in the characteristics of lemon-grass oils from different regions. It is not the purpose of this paper, however, to discuss any questions of systematic relationship or nomenclature of the plant, but since a wide difference has been found in the commercial value of the strains under experimental cultivation, a brief discussion of these will be of interest to the prospective grower.

During the course of the experiments, plants were obtained from a number of sources, and altogether 13 different strains have been tested. Following are the sources of the various strains:

1. Secured from a nursery in Florida. The original stock was from Havana.
2. A local form sold in the Florida nursery trade.
3. Isle of Pines.
4. Porto Rico.
5. Cochin China.
6. Ceylon.
7. Mexico.
8. India.
9. India.
10, 11, and 12. Origin unknown.
13. Ceylon.

These 13 strains fall into the following classes as regards growth characteristics:

(1) The West Indian type, represented by Nos. 1, 2, 3, and 4. The plants are 2½ to 3 feet high, with lax, drooping leaves and of light color.

(2) The East Indian type, represented by Nos. 5, 8, and 9. The plants are 3½ to 4 feet high and erect. The leaves are rather erect and more scabrous than the West Indian form.

(3) The Mexican form, represented by No. 7. This is a weak form, very drooping in habit, with lax leaves and very light in color.

No. 6 has the typical West Indian appearance, but is markedly different in oil yield. No. 13 has the typical East Indian appearance, except the color, which is very light, almost yellowish. Nos. 10, 11, and 12 are of the approved East Indian type.

Table III shows the variations in the yield of oil and the citral content of the oil from these various types for the season of 1915.

It has been found year by year that there is considerable variation in both the yield of oil and the citral content, yet the figures given in Table III may be taken as representative of the varieties

mentioned. It will be noted that the Ceylon forms, Nos. 6 and 13, are very low in oil yield, and the same is true of No. 8, from India.

Both the yield of oil and the citral content of the oil have been found to be affected to a considerable degree by the type of soil on

TABLE III.

Yield and Citral Content of Lemon-grass Oils Distilled from the Various Plants under Cultivation in 1915.

Variety.	Yield of Oil.	Citral Content of the Oil.	Variety.	Yield of Oil.	Citral Content of the Oil.
	Per Cent.	*Per Cent.*		*Per Cent.*	*Per Cent.*
No. 1.....	0.24	80	No. 9.....	0.20	76
No. 5.....	.27	70	No. 10.....	.23	80
No. 6.....	.16	73	No. 11.....	.28	80
No. 7.....	.23	72	No. 12.....	.29	81
No. 8.....	.15	79	No. 13.....	.12	85

which the plants are grown. Therefore, before selecting a variety for commercial planting, tests should be made to determine which variety will give the highest yield of oil per acre and the highest citral content on the land to be used. The vigor of the plants should also be considered, since there seems to be a difference in soil requirements among the varieties tested.

FACTORS AFFECTING THE YIELD OF LEMON-GRASS OIL.

Soil Conditions.—In order to determine the effect of soil conditions on the yield of lemon-grass oil, tests were made in 1908 with the West Indian variety, No. 1, on soils containing various degrees of moisture. On light sandy soil of the high hammock type the yield of oil was 0.31 per cent. and on moinst bottom land 0.27 per cent. Another test on sandy high pine land in a different location gave an oil yield of 0.35 per cent., and on moist land near the lake 0.28 per cent. Further tests with this variety under other conditions of soil moisture gave results which were also much in favor of the sandier and better drained land. In 1915 the plat devoted to the Ceylon variety, No. 6, showed a higher yield of oil from the plants grown on the high, well-drained, sandy soil than from the part of the plat which contained slightly more moisture, 0.16 per cent. being obtained from the former and only 0.11 per cent. from the latter. Similar results were secured in 1914 with varieties Nos. 5, 8 and 9.

The evidence thus far available indicates that for all the forms

of lemon grass tested, a heavy growth of herb with high oil content is to be expected on light, well-drained soil of the high pine type.

Time of Harvest.—Since lemon grass is a perennial crop and two or three cuttings can be made each year, it is of interest to note the difference in yield of oil secured from the plants at each harvest. In Table IV are given the results obtained from each of two harvests for various years.

TABLE IV.

Yield of Lemon-grass Oil Distilled from Plants Harvested at Two Different Times of the Year.

Year and Plants Harvested.	Yield of Oil.		Year and Plants Harvested.	Yield of Oil.	
	First Harvest.	Second Harvest.		First Harvest.	Second Harvest.
1908.	*Per Cent.*	*Per Cent.*	1914—Continued.	*Per Cent.*	*Per Cent.*
First plat.....	0.31	0.33	No. 8.....	0.12	0.38
Second plat.....	.40	.48	No. 9.....	.24	.36
Third plat.....	.20	.35	1915.		
1912.			No. 1.....	.27	.26
No. 1.....	.40	.36	No. 8.....	.11	.11
No. 8.....	.28	.46	No. 9.....	.19	.17
1914.			No. 10.....	.23	.47
No. 1.....	.37	.50	No. 11.....	.28	.40
No. 5.....	.34	.35	No. 12.....	.29	.31
No. 6.....	.16	.20	No. 13.....	.12	.27

These results show that in general the percentage of oil is higher in the second cutting. In the first year of planting, however, the quantity of herb obtained in the second cutting is much less than that from the first cutting; consequently, the acre yield of oil in the first year would be greater from the first cutting rather than from the second.

FACTORS AFFECTING THE CITRAL CONTENT OF LEMON-GRASS OIL.

Closeness of Cutting the Plants.—Experiments conducted with variety No. 1, grown on very light sandy soil, showed that the citral content was highest in the part of the plant nearest the ground. Large plants divided into three portions yielded, on distillation, oil with citral content as follows: Upper portion, 70 per cent.; middle portion, 78 per cent.; and lowest portion, 82 per cent. A similar test made with variety No. 5 divided into only two portions yielded oil with citral content in favor of the lower portion, as follows:

Upper portion, 74 per cent.; lower portion, 76 per cent. These results show that the closest cutting which gives a profitable yield of oil also produces a better quality of oil.

Soil Moisture.—Plants of variety No. 1, grown on soils having varying degrees of moisture, yielded oil with citral content as follows: On dry sandy soil, 75 per cent. citral; on slightly moist sandy loam, 68 per cent.; and on moist loam near the lake, 66 per cent. Further tests with other varieties on different types of soil have given similar results. This would indicate that high citral content can be secured only from plants grown on very well drained soil.

Time of Harvest.—Although the citral content of the oil does not appear to be greatly affected by the time of harvest, the results indicate that of the two harvests each year the oil distilled from plants of the first harvest contains the greater quantity of citral. Data covering a number of years are given in Table V.

TABLE V.

Citral Content of Lemon-grass Oil Distilled from Plants Harvested at Two Different Times of the Year.

Year and Plants Harvested.	Citral Content of Oil.		Year and Plants Harvested.	Citral Content of Oil.	
	First Harvest.	Second Harvest.		First Harvest.	Second Harvest.
1908.	*Per Cent.*	*Per Cent.*	1914—Continued	*Per Cent.*	*Per Cent.*
First plat.....	72	74	No. 8.....	81	72
Second plat.....	74	72	No. 9.....	75	59
Third plat.....	75	72	1915.		
1912.			No. 5.....	70	68
No. 1.....	76	78	No. 6.....	73	71
No. 8.....	78	76	No. 8.....	77	64
1914.			No. 9.....	78	70
No. 1.....	78	..	No. 10.....	80	74
No. 5.....	78	76	No. 11.....	80	82
No. 6.....	77	79	No. 12.....	81	80
			No. 13.....	85	82

SOLUBILITY OF LEMON-GRASS OIL IN ALCOHOL.

For many years it was considered that good lemon-grass oil should be soluble in clear solution in three volumes of 70 per cent. alcohol, and this was the test applied before the method of citral determination was in general use. It served a useful purpose, however, inasmuch as certain adulterations which had become quite

general could thus be detected, but at the present time, when the valuation of the oil is entirely on the basis of the citral content, it is difficult to understand the reason for the continued use of the solubility test. It has been shown repeatedly that in many parts of the world pure lemon-grass oil does not pass the solubility test, especially after it has been stored for several months. This has been true of most of the samples of the oils produced in the Western Hemisphere, so that West Indian lemon-grass oil has come to be a synonym for insoluble oil. This discrimination has kept out of the market many West Indian oils of very high citral content.

There has been much discussion regarding the factors which affect the solubility of the oil, it having been contended that the length of time of distillation is the controlling factor. In order to secure data upon this point the following tests were made: In 1914, 158 pounds of the freshly cut plants were distilled with steam and the oil drawn off in fractions at intervals of 45 and 60 minutes, respectively. The first fraction represented a yield of oil of 0.28 per cent., the citral content of the oil being 80 per cent., while the second fraction represented a yield of 0.04 per cent. of oil, with a citral content of 85 per cent. When first distilled the first fraction gave a slightly cloudy solution with three volumes of 70 per cent. alcohol, but after two months it gave a very cloudy solution in all volumes of 70 per cent. alcohol. The second fraction was soluble with clear solution in three volumes of 70 per cent. alcohol, showing no sign

TABLE VI.

Citral Content and Solubility in 70 Per Cent. Alcohol of Various Fractions of Lemon-grass Oil.

Fractions.	Yield of Oil.	Citral Content of Oil.	Solubility in 70 Per Cent Alcohol.
	Per Cent.	*Per Cent.*	
First 15 minutes...	0.21	39	Soluble with very cloudy solution in two volumes and over.
15 to 30 minutes...	.21	74	Soluble in clear solution in two volumes and over.
30 to 50 minutes...	.05	82	Do.
50 to 90 minutes...	.01	80	Do.

of change after two months. Another sample of 203 pounds of the fresh plants distilled with steam and the oil drawn off in fractions at intervals of 15, 30, 50, and 90 minutes, respectively, gave the results shown in Table VI.

From the results shown in Table VI it is evident that complete extraction of the oil gives a product of greater solubility and higher citral content.

The oils produced in Florida from all varieties of the plant have passed the solubility test when first distilled, but after storing for three months all have become insoluble. At the present time there is a decided tendency to disregard the solubility test, and no difficulty has been encountered in selling the Florida oils at a good price when the citral content was 70 per cent. or more.

COMMERCIAL POSSIBILITIES.

The consumption of lemon-grass oil in the United States for the manufacture of ionone and for perfumery purposes is continually increasing, and it is believed that the demand is sufficient to warrant an attempt to grow the plant for the commercial production of the oil in such parts of the country as possess the proper climatic requirements. Tests on acre plats have been made to determine the cost of production, the best methods of distilling the oil, and the quality of the product. Samples of the oil produced have been sold on the market at the prices prevailing for the better grades of imported oil, and it seems possible to produce the oil commercially at a fair profit.

From the experiments made thus far the following estimates are given of the cost of production and the returns that may be expected for this crop under average conditions:

Expenditures.

First year (per acre) :	
Preparing the land	$ 3.00
Planting	2.00
Fertilizers	8.00
Cultivation	2.00
Harvesting and distilling	5.00
Total expenditures, first year	20.00
Succeeding years (per acre) :	
Cultivation	$ 1.00
Fertilizers	8.00
Harvesting and distilling	8.00
Total expenditures, second year and succeeding years	17.00

Returns.

First year: 25 pounds of oil per acre, at 80 cents $20.00
Succeeding years: 35 pounds of oil per acre, at 80 cents 28.00

In these statements no allowance is made for such charges as taxes, insurance, interest, or depreciation of outfit. It is doubtful whether the production of lemon-grass oil would be profitable if all overhead charges were placed against this crop alone, since the distilling plant would be in use only a few weeks in a year. However, if grown in connection with other volatile-oil plants, so that a long distilling season would be secured, it is believed that this crop will yield returns comparing favorably with other crops grown on the same type of land.

THE PHILADELPHIA DRUG EXCHANGE.

The annual meeting of the Philadelphia Drug Exchange was held on Tuesday, January 23, 1917, in its rooms in the Bourse Building and the work of the year reviewed. Mr. Clayton F. Shoemaker, Chairman of the Committee on Legislation, presented on behalf of the Board of Directors, the fifty-sixth annual report detailing the general conditions of business with special reference to the interests of the drug and chemical trade and the rapid growth in exports by reason of war conditions. Treasurer Anthony M. Hance presented the financial report for the year.

The following officers were elected for 1917: President, John Fergusson; Vice-President, Harry B. French; Secretary, Joseph W. England; Treasurer, Anthony M. Hance; Directors: Charles E. Hires, A. Robinson McIlvaine, Dr. Adolph W. Miller, Harry K. Mulford, Adam Pfromm, Clayton F. Shoemaker, Richard M. Shoemaker and Walter V. Smith.

Addresses were made by Mr. Adam Pfromm, Mr. Walter V. Smith and Mr. Geo. E. Bartol, President of the Philadelphia Bourse. Mr. Alexander C. Ferguson, formerly of Fergusson Bros., who has been actively identified with the drug brokerage and commission business of Philadelphia for more than fifty years, and for a number of years was Secretary of the Drug Exchange, made an address replete with interesting incidents of the development of the Exchange since its organization in 1861. By recent action, the Board of Directors decided against a propaganda for the adoption of the metric

system at this time, by reason of the existing war conditions, urging that action be postponed until the business conditions of the country have again become normal.

The annual dinner was held on Thursday evening, January 25, 1917, at the Bellevue Stratford Hotel, 150 members and guests being present. Mr. Clayton F. Shoemaker acted as toastmaster. The Committee on Entertainment, Mr. Walter V. Smith, Chairman, presented a most enjoyable vocal and instrumental program, while the addresses were of an unusually high character. The speakers were: Dr. John G. Wilson, Superintendent of the Northwest District, Philadelphia Conference, M. E. Church; Mr. Thomas A. Daly, Author of Tom Daly's Column of the Philadelphia Ledger, Mr. Ernest T. Trigg, the recently elected President of the Philadelphia Chamber of Commerce, and Dr. William E. Hughes in an address of "Japan of To-day," illustrated by lantern slides.

At the Annual meeting the death of Ernst T. Fritzsche, senior member of Schimmel & Co., distillers of essential oils and manufacturers of fine chemicals, died on December 21st, at Leipsig, Germany. Mr. Fritzsche was in his sixty-sixth year. Details regarding the life of Mr. Fritzsche are not available, owing to the unusual conditions resulting from the war.

OBITUARY.

Prof. C. Lewis Diehl.

Though the failing health of Professor Diehl had been a matter of concern for several months, his many friends and associates were shocked when the news came of his death on Sunday, March 25th, and thus left another gap in the ranks of the "Old Guard" of Pharmacy's brilliant lights. His long association with the profession and his sincere and continued interest in all matters pertaining thereto, gave him not only a national but an international reputation, and his presence will be missed at the Annual Meetings of the American Pharmaceutical Association and the Kentucky Pharmaceutical Association, where for years his familiar figure was unfailingly in evidence.

C. Lewis Diehl was born at Neustadt, Rhenish Bavaria, Aug. 3, 1840; his father was chief executive in one of the revolutionary districts, and owing to political conditions, was forced to take refuge in France in 1848, from where he emigrated to America in 1849; his wife and three children following him in 1851. The family took up their lives in the New World upon a farm near St. Louis, Mo., where the wife and mother died in 1852 and the farm was abandoned. Young C. Lewis was sent to Oakfield Academy at St. Louis, where he remained for two years, leaving to join his father in Philadelphia, Pa.

At the age of fourteen, he secured his first position with Messrs. R. & G. A. Wright, Perfumers, remaining with them for three years, then going to Chicago. The financial panic of that year (1857) compelled young Diehl to resort to various means of livelihood, but he remained in Chicago until the following year, when he again returned to Philadelphia and became apprenticed to John R. Agney, Spruce and Fifth Sts., Philadelphia; here he laid the foundation of that life's work, to which he gave so much enthusiasm and energy in all his remaining years.

Graduating from the Philadelphia College of Pharmacy in March, 1862, he entered the employ of Messrs. John Wyeth & Bro., assuming charge of their new and extensive manufacturing laboratory. His unflagging energy and unusual attainments were largely instrumental in making the venture successful from the start.

The call of his adopted country was answered by his enlistment in the famous Anderson Cavalry and he remained in the service until the battle of Stone River, where he was severely wounded and was given his discharge.

Joining his father, in Chicago, he remained with him for several months, recuperating from his wounds, only again to enter the Government service, as Assistant Chemist in the United States Army Laboratory at Philadelphia, which position he secured through the recommendations of Messrs. Wyeth & Bro. and the late Prof. John M. Maisch.

The termination of the war being evident, on January 1st, 1865, Mr. Diehl resigned his position and desiring to locate permanently, he went to Chicago with the intention of purchasing a store, but on receiving an offer from the firm of Bender, Mahle & Co. (afterward Mahle & Chappel), he entered their employ only to remain until the following July; leaving to accept the management and re-

organization of the Louisville Chemical Works at Louisville, Ky., a concern originated by Dr. E. R. Squibb and Prof. J. Lawrence Smith, but at that time operated in the interest of Messrs. Wilson, Peter & Co.

The first store owned and operated under his own name was purchased by Mr. Diehl in June, 1869, and was located at First and Walnut Sts., Louisville, Ky., moving in 1874 to Third and Broadway, where he continued in business until his retirement to private life (1904).

Joining the American Pharmaceutical Association in 1863 at the Baltimore meeting, he attended his first meeting at Detroit in 1866, when he was elected Chairman of the Committee on Progress of Pharmacy, a position to which he was re-elected in 1867. In 1871 Professor Diehl was elected First Vice President of the Association and at the Louisville meeting in 1874 he became its President.

A volunteer report on the Progress of Pharmacy submitted in 1872 met with so much approbation and commendation that the Association elected him to the newly created office Reporter on Progress of Pharmacy, a position which he occupied almost continuously until the San Francisco meeting of 1915; when failing health rendered his retirement necessary.

The Louisville College of Pharmacy owes its organization to Professor Diehl and others whom he interested in it; he was its first President and from 1870 to 1881 continued to preside over its destinies; he also occupied the chair of Pharmacy until 1886, with the exception of the sessions of 1881–2 and 1882–3; resigning on account of a throat affection.

His Alma Mater, the Philadelphia College of Pharmacy, conferred the richly merited degree Master in Pharmacy upon him in 1887.

The organization of the Kentucky Board of Pharmacy was largely owing to his unfailing energy, and he was a member of that body for the first six years of its existence and afterward at frequent intervals until the time of his death.

His Chairmanship of the National Formulary Revision Committee, his association on the U. S. P. Revision Committee and other pharmaceutical honors would fill more space than is allotted in this brief sketch, but it is to be hoped that a complete list of his published works will be searched out and tabulated.